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REVIEW

Mechanisms of osteolytic and osteoblastic skeletal lesions

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The bone is a frequent site for tumor metastasis, and cancer in the bone results in marked disturbances of bone remodeling that can be lytic, blastic or a combination of the two. Patients with advanced malignancies that have metastasized to the bone frequently suffer from debilitating skeletal-related events, including pathologic fractures, spinal cord compression syndromes, disorders of calcium and phosphate homeostasis and severe cancer-related pain. This review will discuss recent studies on the mechanisms responsible for osteolytic and osteoblastic metastasis and how their identification has resulted in the development of new agents for patients with metastatic bone disease.

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Introduction

The bone is one of the most frequent sites for tumor metastasis, with over 450 000 patients in the United States suffering from cancer in bone (CIB).[1] Bone is the preferred site for breast (BCa) and prostate cancer (PCa) metastases, but many other cancers, including hematologic malignancies such as multiple myeloma (MM), can also colonize the skeleton. MM is the most frequent cancer to involve bone, and, 70% of MM patients have skeletal lesions at diagnosis, and over 90% develop bone lesions over their disease course.[2] Virtually, all patients who die from advanced PCa or MM, and the majority who die from advanced BCa, have tumor-induced bone lesions, and CIB is the major cause of cancer-related pain in patients with advanced malignancies. Tumor metastasis to bone results in marked disturbance of bone remodeling and imbalances in osteoclast (OCL) and osteoblast (OB) numbers and activity,[1] leading to debilitating skeletal-related events with catastrophic sequelae including excruciating bone pain, pathologic fractures, spinal cord and nerve compression syndromes, derangements of calcium and phosphate homeostasis and diminished quality of life. For the purpose of radiologic classification, bone metastatic lesions can be broadly divided into those that are purely lytic, as in MM, primarily lytic or mixed, as in BCa, where as many as 25% of patients with bone lesions also have osteoblastic lesions,[3] and primarily blastic lesions, as in PCa (although patients with PCa bone metastasis can have very high bone resorption NTX marker levels[3]). Associated local bone formation reactions frequently occur adjacent to bone metastases from breast and PCa and can be visualized on technetium-bone scans.

Importantly, once cancers involve bone, the majority of patients are currently incurable. Thus, new mechanistic-based therapies are needed to improve outcomes for patients with CIB. This chapter will review recent studies on the mechanisms responsible for osteolytic and osteoblastic metastasis and how their identification has resulted in the development of new agents for CIB patients.

Bone as the Preferred Site for Metastasis

As noted above, bone is a frequent site of metastases. Multiple extrinsic and intrinsic factors contribute to the homing of tumor cells to bone. Kang and coworkers have found that expression of only 3–4 genes in BCa cells is required for the cancer cells to preferentially metastasize to the bone. These include interleukin-11 (IL-11), which encodes an osteolytic factor, connective tissue growth factor (CTGF), which encodes an angiogenic factor, the membrane receptor/adhesive proteins CXCR4 and osteopontin and the metalloproteinase MMP1. Breast cancer cells that overexpressed IL-11, osteopontin and either CXCR4 or CTGF displayed the highest bone metastatic potential. Further, these bone metastatic factors could be upregulated by transforming growth factor-β (TGF-β), which is released and activated by osteoclastic bone resorption stimulated by tumor cells in the bone microenvironment.[4] Recently, Lu *et al.*[5] found that acquisition of VCAM-1 expression in quiescent BCa cells in the bone induced their transition from dormant micrometastases to active osteolytic metastases, with increased tumor cell growth, enhanced metastasis to bone and osteolysis.

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Tumor cells at primary sites can affect cells in the bone microenvironment, enhancing the capacity of tumor cells to seed the bone and secreting a multitude of chemokines that direct the migration, proliferation and differentiation of tumor cells to sites that support their growth. Stephen Paget originally proposed the 'seed and soil' hypothesis, in which tumor cells preferentially metastasize to sites such as the bone because of specific properties of the metastatic site as opposed to sites contiguous to the primary tumor.[6]

Tumor-derived exosomes have been shown to have an important role in preparing the microenvironment for tumor metastases. Exosomes, vesicles derived from the intravesicular membranes of cells that are used for the packaging and transport of cytosolic proteins and other factors from these cells to distant sites via the blood stream and other biologic fluids, fuse to the membranes of cells at these sites, allowing the contents of the exosomes to be transferred to the cells. Lyden and colleagues demonstrated that melanoma-derived exosomes can influence metastasis at both typical and atypical sites, including the lungs, lymph nodes, mesentery and brain, by educating bone marrow cells.[7] Roccaro *et al.* reported that exosomes released by MM patient bone marrow stromal cells enhanced the growth of myeloma cells *in vitro* and *in vivo*. In contrast, normal bone marrow stromal cell-derived exosomes suppressed myeloma cell growth and contained higher levels of the tumor suppressor microRNA miR-15a, as compared with exosomes from myeloma patients.[8]

Other cellular products may also facilitate bone metastasis. Tumor-derived syndecan-1 can regulate tumor growth and metastasis[9] and enhance osteoclastogenesis at distant sites. BCa cells implanted in the mammary fat pad of mice express heparanase, which cleaves syndecan-1 from the surface of tumor cells, allowing binding of the shed syndecan-1 to induce macrophage and marrow endothelial cell-derived IL-8 that increases osteoclastogenesis.[10]

Adhesive molecules expressed on tumor cells and cellular products from tumor cells also increase the receptivity of the marrow microenvironment to metastasis. Shiozawa *et al.*[10] reported in models of PCa bone metastasis that human PCa cells target the normal hematopoietic stem cell niches and directly compete with hematopoietic stem cells for the niche. Expression of the adhesion molecules Annexin A2 (AXA2) and CCL12 (SDF-1) on marrow stromal cells and OBs in the endosteal hematopoietic stem cell niche allows hematopoietic stem cells and PCa cells expressing CXCR4 and the AXA2 receptor to bind and home to the marrow. Importantly, when cancer cells home to the stem cell niche, they displace hematopoietic stem cells and induce hematopoietic stem cells to terminally differentiate.

Bone Remodeling in Osteolytic Metastasis

Normally, bone resorption and formation are tightly linked, with bone formation occurring at discrete sites of previous bone resorption. This 'coupled' bone remodeling is largely dependent on communication between OCLs and OBs[4] via bidirectional signaling between Eph receptors expressed on OBs bone marrow stromal cells and ephrins on OCLs[11] (**Figure 1**). In osteolytic metastasis and MM, the bone remodeling process is imbalanced, or uncoupled, with increased osteoclastic bone resorption driven by OCL-activating factors produced by the tumor cells themselves or by cells in the bone microenvironment in response to the tumor cells. The mechanism by which tumor cells access cells in the bone and alter the surface of a bone-remodeling site is emerging. Andersen and co-workers[12] have demonstrated that, under physiologic conditions, a vascular canopy of flat cells expressing OB markers covers bone-remodeling sites. Osteolytic tumors, such as MM, disrupt the bone remodeling compartment, allowing exchange of soluble factors as well as direct cell-to-cell contact between MM cells and bone cells. Thus, physical exchange of cytokines and other factors between tumor cells and bone cells occurs and mediates the bone destruction and deficient bone formation characteristic of osteolytic bone lesions.

Bone Remodeling in Osteoblastic Metastases

Unlike bone metastases from MM or BCa, bone lesions in PCa are classified as osteoblastic, primarily due to their characteristic osteosclerotic appearance on x-ray or computed tomography scan. Serum levels of OB proliferation markers, including bone-specific alkaline phosphatase and type I procollagen C-propeptide[13], are elevated in PCa patients with bone disease; however, new bone formation at metastatic sites is immature and of poor quality, and lesions are associated with an elevated osteoid volume, surface area and mineral apposition. PCa cells secrete factors that directly and indirectly alter the OB function and express factors critical for normal bone development and remodeling, including bone morphogenetic proteins (BMPs), TGF-β, platelet-derived growth factor, adrenomedullin, insulin-like growth factor (IGF-1), fibroblast growth factor and vascular endothelial growth factor (VEGF).[14]

Interestingly, the osteolytic factor, PTHrP, is abundantly expressed in PCa metastases, despite the fact that these lesions are primarily blastic. Liao and colleagues demonstrated that PTHrP increases OB progenitor cell proliferation and induces early OB differentiation.[15] Others have noted the strong homology between PTHrP NH2-termina fragments and endothelin 1 (ET1), which promotes OB growth at metastatic sites and thereby stimulates bone formation via activation of the ET_AR.[16] ET1 has also been shown to activate the Wnt signaling pathway and to suppress DKK1.[17]

Other PCa cell-derived factors, including prostate-specific antigen (PSA), a kallikrein serine protease and urokinase-type plasminogen activator (uPA), modify the bone microenvironment.[18]PSA cleaves PTHrP and activates TGF-β, an OB growth factor. uPA aids in degradation of the extracellular matrix, facilitating tumor cell invasion, and also cleaving and activating TGF-β, and stimulates OB proliferation by hydrolyzing IBF-binding proteins (IGFBPs).[18]

The Vicious Cycle Hypothesis

The 'vicious cycle' hypothesis (**Figure 2**) proposes that tumor cells in bone stimulate osteoclastic bone resorption by secreting factors that directly or indirectly induce OCL formation. Upregulation of RANK ligand (RANKL) in OB precursors increases OCL activity via release of cytokines by tumor cells that increase RANKL production by bone marrow stromal cells, through adhesive interactions between marrow cells and tumor

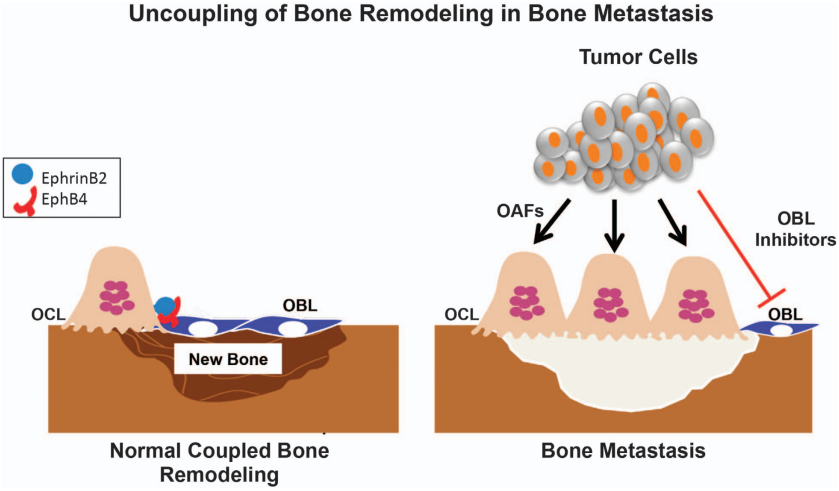

Figure 1 Uncoupled bone remodeling in bone metastasis. Physiologic (nonmalignant) bone remodeling, (left) is a coupled process, with balanced osteoclast (OCL) and osteoblast (OBL) activity. Bidirectional signaling between ephrin B2, found on OCL, and EphB4, found on BMSC and OB, maintains this balance, negatively regulating OCL formation and promoting osteoblast differentiation. The bone remodeling process in metastatic bone disease, (right), and MM is uncoupled, and ephrin B2 and EphB4 expressions are downregulated in MMBD. In addition, tumor cells produce osteoclast-activating factors (OAFs) and osteoblast inhibitor factors.

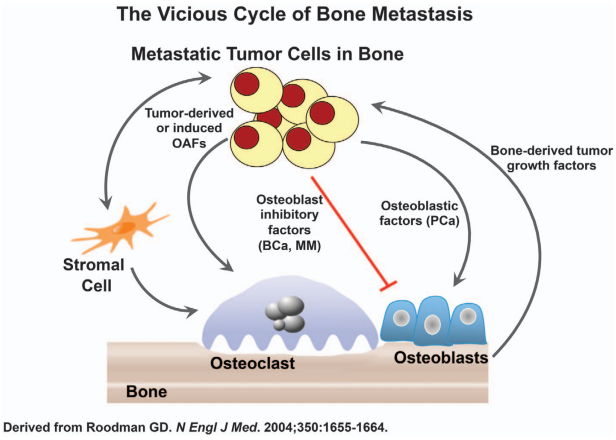

Figure 2 The vicious cycle of bone metastasis. Tumor cells in bone that induce osteolytic metastases (breast cancer, BCa and multiple myeloma, MM) secrete osteolytic factors (osteoclast-activating factors, OAFs) and osteoblast inhibitory factors. Growth factors, such as TGF-β, are released from the bone matrix as a result of osteoclastic bone resorption. Tumor cells that result in osteoblastic bone metastases such as prostate cancer (PCa) secrete factors that induce osteoblastic proliferation and differentiation, including VEGF, PDGF and ET1. Myeloma cells produce growth factors that stimulate the growth of bone marrow stromal cells, which in turn produce OAFs, such as IL-6, macrophage colony-stimulating factor, TNFα and RANKL, increasing the ratio of RANKL:OPG, which enhances osteoclast formation. Bone marrow stromal cells also produce soluble factors that enhance tumor growth.

cells (**Figure 3**) and direct stimulation of osteoclast precursor differentiation by cytokines and chemokines released by tumor cells. Osteolytic tumors, such as MM, also produce factors that inhibit bone formation, further exacerbating the bone destructive process. The increased bone resorbing activity that occurs with CIB releases and activates immobilized growth factors from the bone matrix, such as TGF-β and other growth factors, that stimulate the growth of tumor cells.[19] Some tumors, in particular PCa, also produce OB-stimulating factors that increase bone formation. Release of cytokines and chemokines from the increased numbers of OBs further enhances tumor growth. Importantly, the early events that allow disseminated tumor cells to colonize the bone remain incompletely understood. Recent work defining the mechanism by which disseminated BCa cells transition to micrometastases has demonstrated that the microenvironmental niche occupied by these microscopic bone lesions is primarily composed of OB lineage cells, which promote tumor cell proliferation via heterotypic adherins junctions that activate mTOR,[20] suggesting an intermediate stage between solitary disseminated tumor cells and osteolytic metastasis, which warrants further investigation as a potential therapeutic target for the prevention of bone metastasis.

OCL-activating Factors

Over the last decade, multiple OCL stimulatory factors and OB inhibitory factors produced or induced by tumor cells in the bone have been identified that regulate OCL and OB activity in osteolytic metastases and myeloma. RANKL is the central regulator of OCL formation, activity and survival. Hematopoietic precursor cells exposed to macrophage colony-stimulating factor (M-CSF) express receptors for activator of NFκB (RANK), which binds RANKL and induces differentiation into active, multinucleated OCLs. The effects of RANKL are opposed by osteoprotegerin (OPG), its soluble decoy receptor. Blockade of the RANKL pathway with the neutralizing antibody, denosumab, effectively blocks the formation of OCLs and subsequent bone destruction.

RANKL is produced in soluble and membrane-anchored form by cells in the early OB lineage and osteocytes, the most abundant bone cell and the major source of RANKL in the bone, in response to osteolytic factors such as IL-11, parathyroid hormone-related protein, PTHrP, the primary causal agent of hypercalcemia of malignancy and high concentrations of 1,25-dihydroxyvitamin D. In addition, multiple RANKL-independent OCL stimulator factors including ILs 3, −8, −6, −17, −18, macrophage-induced protein MIP-1a and Activin A are produced or induced by CIB.[21]

MIP-1α is a chemokine produced by MM cells in 70% of patients that is a potent inducer of human OCL formation. *MIP-1α* gene expression is highly correlated with bone

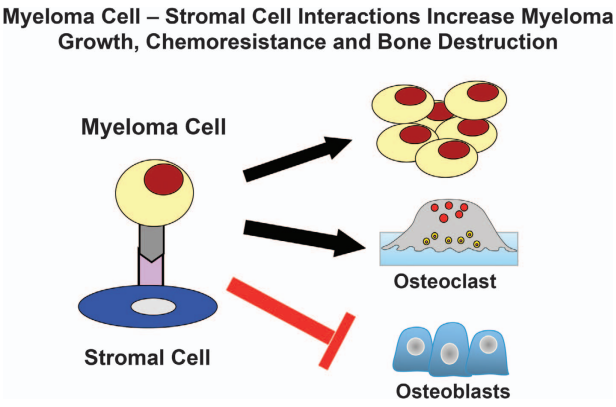

Figure 3 Myeloma cell– stromal cell adhesive interactions increase myeloma growth, chemoresistance and bone destruction. Myeloma cells induce production of RANKL by bone marrow stromal cells through adhesive interactions between vascular cell adhesion molecule-1 (VCAM-1) on marrow stromal cells and $\alpha_4\beta_1$ integrin (very late antigen 4, VLA-4) on myeloma cells. Other osteoclast-activating factors, such as MIP-1α, also enhance myeloma cell adhesion to bone marrow stromal cells, further enhancing marrow stromal cell production of osteoclast-activating factors (OAFs). OCL and stromal cell-derived tumor-activating factors such as IL-6 and TNFα also increase the growth of myeloma cells. Myeloma cells also produce factors that suppress OBL differentiation, such as sclerostin, DKK1, hepatocyte growth factor (HGF), IL-3, IL-7 and TNFα.

destruction in MM, and elevated levels of MIP-1α in MM patients correlate with an extremely poor prognosis.[22] MIP-1α acts as a chemotactic factor for OCL precursors and can induce differentiation of OCL progenitors, contributing to OCL formation independent of RANKL.[23,24] In addition, MIP-1α potentiates both RANKL and IL-6 stimulated OCL formation.[25] MIP-1α induces OCL formation and bone destruction in murine models of MM and blocking MIP-1α expression in MM cells injected into severe combined immunodeficiency (SCID) mice or treating the animals with a neutralizing antibody to MIP-1α decreased tumor burden and bone destruction.[23] MIP-1α also enhances homing of MM cells to the bone marrow through increased adhesive interactions between MM cells and marrow stromal cells by increasing expression of β_1 integrins on MM cells. This results in increased production of RANKL, IL-6, VEGF and tumor necrosis factor-α (TNFα) by marrow stromal cells, which further enhances MM cell growth, angiogenesis and bone destruction.

TNFα is a bifunctional cytokine that can induce OCL formation and suppress OB differentiation through its effects on Runx2 and Gfi-1 expression in marrow stromal cells (see below). TNFα is induced by adhesive interactions between MM cells and marrow stromal cells in both MM cells and BMSC and can increase IL-6 production by marrow stromal cells.

IL-3 is another OCL stimulatory factor that is significantly elevated in the BM plasma of 70% of MM patients.[26] IL-3 induces OCL formation in human bone marrow cultures at levels similar to those present in MM patient samples. OCL formation induced by marrow plasma from MM patients can be inhibited by using a blocking antibody to IL-3. IL-3 also indirectly induces osteoclastogenesis by enhancing the effects of RANKL and MIP-1α on the growth and development of OCLs and stimulates MM cell growth directly[26] and inhibits OBL formation through a factor produced by macrophages in the marrow microenvironment. IL-3 also induces Activin A expression by MM patient bone marrow macrophages, which mediates the osteoclastogenic effects of IL-3 *in vivo* and *in vitro*. Similar to IL-3, Activin A can also inhibit OB differentiation in MM patients.[27]

AXII is produced by stromal cells and OCL and increases OCL formation, hematopoietic stem cell mobilization and homing of PCa cells to the bone.[28] AXII is upregulated in MM, and, MM-derived AXII increases proliferation of MM cell lines, possibly through an autocrine mechanism. AXII secreted by OCL and stromal cells enhances the growth of MM cells in the bone marrow by binding to the AXII receptor on MM cells, primarily through a paracrine mechanism. In addition, AXII induces stromal cell production of RANKL, further stimulating OCL formation.[29]

TGF-β is another factor upregulated in bone metastasis that has a multiplicity of effects in the tumor–bone microenvironment. TGF-β is produced by many types of tumor cells and released in the bone microenvironment by enhanced osteoclastic bone resorption. TGF-β can increase production of PTHrP by tumor cells and drive the epithelial-mesenchymal transition of BCa to enhance their metastatic potential.[30] Further, TGF-β can increase production of IL-6, IL-11 and VEGF by tumor cells, which enhance bone metastasis and OCL formation,[31] and Jagged1, a downstream effector of the TGF-β pathway that promotes osteolytic bone metastasis.[32]

OB Inhibitors in Bone Metastasis and Myeloma

Multiple OB inhibitors are produced in MM, including the Wnt inhibitors DKK1 and secreted frizzled-related protein 2 (sFRP2), IL-7 and sclerostin. DKK1 is produced by primary CD138 + MM cells and is upregulated in the marrow of patients with BCa and PCa bone metastasis. (sFRP2, another Wnt inhibitor produced by MM cells, also suppresses OB differentiation in MM.)

DKK1 inhibits osteoblastogenesis by sequestering low-density lipoprotein receptor-related protein (LRP) 5/6 from binding Wnt, ultimately downregulating RUNX2 activity,[33,34] but can also indirectly enhance osteoclastogenesis by increasing OBL expression of OPG[35] and downregulating RANKL expression.[36] *In vitro* evaluation of an anti-DKK1 antibody in the SCID-hu murine MM model resulted in growth inhibition of MM cell growth and increased bone formation in the implanted fetal bone,[37] and a phase I/II trial of anti-DKK1 has been completed in patients with MM. These studies indicate that DKK1 is a key regulator of bone remodeling in both physiological and pathological conditions and that blocking this factor may inhibit both stimulation of osteoclastogenesis and inhibition of OBL in myeloma bone disease. Interestingly, DKK1 mRNA and serum levels correlate with MM bone disease in patients.[38]

IL-7 is another potential inhibitor of OBL differentiation in MM that induces RANKL production by T-lymphocytes and mediates MM-induced OBL inhibition by downregulating RUNX2 transcriptional activity.[39] Weitzmann *et al.*[40] reported that IL-7 levels were increased in sera of mice in an ovariectomy model of postmenopausal osteoporosis and that IL-7 blocked new bone formation after ovariectomy. Addition of IL-7 blocked both basal and BMP2-stimulated OB activity in mouse calvarial OB cultures. Giuliani *et al.*[41] reported that IL-7 levels were increased in the marrow of MM patients and that IL-7 inhibited both early and late human OB precursor differentiation in a

dose-dependent manner. Recently, D'Souza *et al.*[42] reported that TNF-α and IL-7 induce Gfi-1, a transcriptional repressor of RUNX2. Gfi-1 is increased in stromal cells from patients and directly interacts with the RUNX2 promoter to block RUNX2 expression. In addition, Gfi-1 can bring histone deacetylases and other co-repressors to the RUNX2 promoter and may contribute to the long-term suppression of OBL activity present in MM patients.

Sclerostin levels are increased in patients with MM, and sclerostin has been reported to be produced by MM cells.[43] Sclerostin is usually produced by osteocytes and suppresses Wnt signaling in OB precursors to block OB differentiation. A neutralizing antibody to sclerostin has been developed and is in clinical trial for osteoporosis.

Osteocytes and Bone Metastasis

Osteocytes comprise more than 95% of the cells in bone. Although embedded into the bone matrix, osteocytes are closely connected to cells on the bone surface, the bone marrow and each other via the osteocytic lacunar-cannalicular network. Osteocyte-derived molecules regulate OB and OCL formation and activity. Specifically, osteocytes express sclerostin, an OB inhibitor, and RANKL, M-CSF and OPG, cytokines that regulate osteoclastogenesis and bone resorption. Apoptotic osteocytes also affect bone homeostasis through the recruitment of OCL precursors, thereby targeting bone resorption to specific areas of the bone. However, the contribution of osteocytes to bone metastasis and the skeletal effects of CIB is relatively unexplored. Sottnik *et al.*[44] reported that tumor-induced pressure in the bone microenvironment in PCa bone metastasis upregulated CCL5 and matrix metalloproteinases in osteocytes, which enhanced tumor growth in bone. Giuliani and co-workers reported that apoptotic osteocytes are increased in patients with myeloma and that apoptotic osteocytes produced increased levels of IL-11 that can stimulate OCL formation.[45] In addition, osteocytes from patients with bone lesions expressed higher levels of IL-11 than those without bone lesions. We recently found that direct cell-to-cell interaction between MM cells and osteocytes occurred in an *in vivo* model of MM and that MM cells activate osteocytic Notch signaling to trigger osteocyte apoptosis. Direct MM-osteocyte contact also induced profound changes in osteocytic gene expression, upregulating *Sost* (the gene encoding sclerostin), decreasing Wnt signaling and downregulating OPG. In addition, MM cell-derived soluble factors upregulate osteocytic RANKL, thus increasing the RANKL/OPG ratio.[46] These findings suggest that MM-osteocyte interactions have a major role in the skeletal effects of MM and that osteocytes have an important role in the metastatic niche.

Role of Immune Cells in Bone Metastasis

Until recently, the vicious cycle hypothesis did not account for immune cells present in the marrow, including mesenchymal stromal cells, hematopoietic cells, T cells, B cells and macrophage-derived cells, which can stimulate bone destruction or enhance tumor cell homing to bone.

T cells, marrow stromal cells, myeloid suppressor cells and dendritic cells have major roles in regulating tumor metastasis to the bone and the growth of myeloma in bone. Dendritic cells and myeloid suppressor cells have been reported to differentiate into OCLs in the presence of MM cells in the bone. Th17 T cells are an important subset of CD4 + T-helper cells that contribute to the growth of CIB. In normal bone marrow, the population of Th1 cells (interferon-producing T cells) that can protect patients with cancer from metastasis[47] exceeds the population of Th17 cells (IL-17-producing T cells). However, in myeloma, the ratio of Th1 to Th17 cells in the bone marrow is reversed, and there is a 10-fold excess of Th17 cells compared with Th1 cells.[48] This change in T-cell subset distribution creates a microenvironment that enhances OCL activation. Dhodapkar *et al.*[49] reported that dendritic cells in the myeloma bone marrow microenvironment mediate the induction of Th17 cells and that IL-6, IL-23 and IL-1 are also involved in dendritic cell-mediated expansion of Th17 cells in myeloma. The increase in IL-17 levels in the marrow also supports myeloma cell growth and increases OCL formation, as IL-17 can induce RANK ligand production by T cells. Further, Lin and coworkers demonstrated that TNFα, IL-6 and IL-17 generated by host immune cells or tumor cells also led to loss of anti-tumor immunity and enhanced tumor growth. IL-17 is also a growth factor for PCa cells[50] and has a role in bone metastasis from BCa.[51] Immune cells can also produce factors that stimulate angiogenesis and prepare the pre-metastatic niche for bone metastasis and serve as OCL precursors. Mesenchymal stromal cells produce large amounts of IL-6 that enhances the growth and prevents apoptosis of myeloma cells, and stimulates OCL formation, as well as produce RANK ligand. Finally, immune cell–tumor cell interactions that suppress anti-tumor immune responses are an emerging therapeutic target for modulating the growth of tumors. Clinical trials targeting immune checkpoints such as PD-1 and PD-L1 interactions that can block the growth of tumors have recently begun for a number of tumor types. In addition, antibodies to cytokines and small molecule inhibitors to receptors for cytokines produced by immune cells and mesenchymal stromal cells that enhance both tumor growth and bone destruction are in development.

Role of MicroRNAs in Bone Metastasis

Altered expression of tumor cell microRNAs (miRNAs), small non-coding RNAs that regulate post-transcriptional gene expression, has also been linked to BCa and PCa bone metastasis. Increased expression of *Cbfa1*, the master gene controlling OB differentiation, has been linked to BCa and PCa bone metastasis. In BCa cells, increased Cbfa1 expression is caused by downregulation of miRNAs 135 and 203, which normally suppress *Cbfa1* gene expression, enhancing the capacity of BCa cells to metastasize to the bone.[52] miR-135 or miR-203 also suppresses Runx2 expression in BCa and PCa cells. Reconstitution of miR-135 or miR-203 in MDA-MB-231 BCa cells impaired their ability to grow in the bone and induce bone resorption.[52] miRNA 135 and 203 replacement using synthetic oligonucleotides suppressed both spontaneous metastasis to the bone and tumor growth in the bone environment. In addition, downregulation of miR-135 or miR-203 enhances the capacity of BCa cells to metastasize to the bone. Ell *et al.*[53] demonstrated that increased expression of miR-141 and 219, which are downregulated in OCLs by BCa cells in bone, blocked osteoclastic bone resorption stimulated

by tumor cells. These results suggest that miRNA delivery is a viable therapeutic strategy to disable the metastatic disease progression.

Summary

Multiple mechanisms contribute to bone metastasis and make bone a preferential site for metastasis. Several mechanisms and substances that mediate the effects of CIB are now being developed as therapeutic targets for treating patients with CIB. These treatments include novel antibodies, immune modulation of tumor growth and miRNAs. Hopefully, with the rapid progress in research on the mechanisms responsible for the effects of CIB, patients with CIB will no longer be incurable.

Conflict of Interest

GDR is a consultant for Amgen. RS has no competing financial interests to disclose.

References

1. Roodman GD. Mechanisms of bone metastasis. *N Engl J Med* 2004; **350**: 1655–1664.
2. Greenberg AJ, Rajkumar SV, Therneau TM, Singh PP, Dispenzieri A, Kumar SK. Relationship between initial clinical presentation and the molecular cytogenetic classification of myeloma. *Leukemia* 2014; **28**: 398–403.
3. Coleman RE. Skeletal complications of malignancy. *Cancer* 1997; **80**: 1588–1594.
4. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C *et al.* A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 2003; **3**: 537–549.
5. Lu X, Mu E, Wei Y, Riethdorf S, Yang Q, Yuan M *et al.* VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging alpha4beta1-positive osteoclast progenitors. *Cancer Cell* 2011; **20**: 701–714.
6. Paget S. The distribution of secondary growths in cancer of the breast. *Lancet* 1889; **1**: 571–573.
7. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G *et al.* Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med* 2012; **18**: 883–891.
8. Roccaro AM, Sacco A, Maiso P, Azab AK, Tai YT, Reagan M *et al.* BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. *J Clin Invest* 2013; **123**: 1542–1555.
9. Sanderson RD, Yang Y. Syndecan-1: a dynamic regulator of the myeloma microenvironment. *Clin Exp Metastasis* 2008; **25**: 149–159.
10. Shiozawa Y, Pedersen EA, Havens AM, Jung Y, Mishra A, Joseph J *et al.* Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. *J Clin Invest* 2011; **121**: 1298–1312.
11. Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T *et al.* Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab* 2006; **4**: 111–121.
12. Andersen TL, Sondergaard TE, Skorzynska KE, Dagnaes-Hansen F, Plesner TL, Hauge EM *et al.* A physical mechanism for coupling bone resorption and formation in adult human bone. *Am J Pathol* 2009; **174**: 239–247.
13. Jung K, Lein M, Stephan C, Von Hosslin K, Semjonow A, Sinha P *et al.* Comparison of 10 serum bone turnover markers in prostate carcinoma patients with bone metastatic spread: diagnostic and prognostic implications. *Int J Cancer* 2004; **111**: 783–791.
14. Guise TA, Mohammad KS, Clines G, Stebbins EG, Wong DH, Higgins LS *et al.* Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clinical cancer research: an official journal of the American Association for Cancer Research 2006; **12**: 6213s–6216ss.
15. Liao J, Li X, Koh AJ, Berry JE, Thudi N, Rosol TJ *et al.* Tumor expressed PTHrP facilitates prostate cancer-induced osteoblastic lesions. *Int J Cancer* 2008; **123**: 2267–2278.
16. Schluter KD, Katzer C, Piper HM. A N-terminal PTHrP peptide fragment void of a PTH/PTHrP-receptor binding domain activates cardiac ET(A) receptors. *Br J Pharmacol* 2001; **132**: 427–432.
17. Clines GA, Mohammad KS, Bao Y, Stephens OW, Suva LJ, Shaughnessy Jr. JD *et al.* Dickkopf homolog 1 mediates endothelin-1-stimulated new bone formation. *Mol Endocrinol* 2007; **21**: 486–498.
18. Ibrahim T, Flamini E, Mercatali L, Sacanna E, Serra P, Amadori D. Pathogenesis of osteoblastic bone metastases from prostate cancer. *Cancer* 2010; **116**: 1406–1418.
19. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer* 2002; **2**: 584–593.
20. Wang H, Yu C, Gao X, Welte T, Muscarella AM, Tian L *et al.* The osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. *Cancer cell* 2015; **27**: 193–210.
21. Roodman GD. Genes associate with abnormal bone cell activity in bone metastasis. *Cancer Metastasis Rev* 2012; **31**: 569–578.
22. Hashimoto T, Abe M, Oshima T, Shibata H, Ozaki S, Inoue D *et al.* Ability of myeloma cells to secrete macrophage inflammatory protein (MIP)-1alpha and MIP-1beta correlates with lytic bone lesions in patients with multiple myeloma. *Br J Haematol* 2004; **125**: 38–41.
23. Choi SJ, Oba Y, Gazitt Y, Alsina M, Cruz J, Anderson J *et al.* Antisense inhibition of macrophage inflammatory protein 1-alpha blocks bone destruction in a model of myeloma bone disease. *J Clin Invest* 2001; **108**: 1833–1841.
24. Oyajobi BO, Franchin G, Williams PJ, Pulkrabek D, Gupta A, Munoz S *et al.* Dual effects of macrophage inflammatory protein-1alpha on osteolysis and tumor burden in the murine 5TGM1 model of myeloma bone disease. *Blood* 2003; **102**: 311–319.
25. Han JH, Choi SJ, Kurihara N, Koide M, Oba Y, Roodman GD. Macrophage inflammatory protein-1alpha is an osteoclastogenic factor in myeloma that is independent of receptor activator of nuclear factor kappaB ligand. *Blood* 2001; **97**: 3349–3353.
26. Lee JW, Chung HY, Ehrlich LA, Jelinek DF, Callander NS, Roodman GD *et al.* IL-3 expression by myeloma cells increases both osteoclast formation and growth of myeloma cells. *Blood* 2004; **103**: 2308–2315.
27. Silbermann R, Bolzoni M, Storti P, Guasco D, Bonomini S, Zhou D *et al.* Bone marrow monocyte-/macrophage-derived activin A mediates the osteoclastogenic effect of IL-3 in multiple myeloma. *Leukemia* 2014; **28**: 951–954.
28. Shiozawa Y, Havens AM, Jung Y, Ziegler AM, Pedersen EA, Wang J *et al.* Annexin II/Annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. *J Cell Biochem* 2008; **370**: 370–380.
29. D'Souza S, Kurihara N, Shiozawa Y, Joseph J, Taichman R, Galson DL *et al.* Annexin II interactions with the annexin II receptor enhance multiple myeloma cell adhesion and growth in the bone marrow microenvironment. *Blood* 2012; **119**: 1888–1896.
30. Deckers M, van Dinther M, Buijs J, Que I, Lowik C, van der Pluijm G *et al.* The tumor suppressor Smad4 is required for transforming growth factor beta-induced epithelial to mesenchymal transition and bone metastasis of breast cancer cells. *Cancer Res* 2006; **66**: 2202–2209.
31. Kang Y, He W, Tulley S, Gupta GP, Serganova I, Chen CR *et al.* Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. *Proc Natl Acad Sci USA* 2005; **102**: 13909–13914.
32. Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell* 2011; **19**: 192–205.
33. Gaur T, Lengner CJ, Hovhannisyan H, Bhat RA, Bodine PV, Komm BS *et al.* Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. *J Biol Chem* 2005; **280**: 33132–33140.
34. Takada I, Mihara M, Suzawa M, Ohtake F, Kobayashi S, Igarashi M *et al.* A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-gamma transactivation. *Nat Cell Biol* 2007; **9**: 1273–1285.
35. Glass 2nd DA , Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H *et al.* Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005; **8**: 751–764.
36. Spencer GJ, Utting JC, Etheridge SL, Arnett TR, Genever PG. Wnt signalling in osteoblasts regulates expression of the receptor activator of NFkappaB ligand and inhibits osteoclastogenesis in vitro. *J Cell Sci* 2006; **119**: 1283–1296.
37. Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy Jr. JD . Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. *Blood* 2007; **109**: 2106–2111.
38. Kaiser M, Mieth M, Liebisch P, Oberlander R, Rademacher J, Jakob C *et al.* Serum concentrations of DKK-1 correlate with the extent of bone disease in patients with multiple myeloma. *Eur J Haematol* 2008; **80**: 490–494.
39. Giuliani N, Colla S, Morandi F, Lazzaretti M, Sala R, Bonomini S *et al.* Myeloma cells block RUNX2/CBFA1 activity in human bone marrow osteoblast progenitors and inhibit osteoblast formation and differentiation. *Blood* 2005; **106**: 2472–2483.
40. Weitzmann MN, Roggia C, Toraldo G, Weitzmann L, Pacifici R. Increased production of IL-7 uncouples bone formation from bone resorption during estrogen deficiency. *J Clin Invest* 2002; **110**: 1643–1650.
41. Giuliani N, Rizzoli V. Myeloma cells and bone marrow osteoblast interactions: role in the development of osteolytic lesions in multiple myeloma. *Leuk Lymphoma* 2007; **48**: 2323–2329.
42. D'Souza S, del Prete D, Jin S, Sun Q, Huston AJ, Kostov FE *et al.* Gfi1 expressed in bone marrow stromal cells is a novel osteoblast suppressor in patients with multiple myeloma bone disease. *Blood* 2011; **118**: 6871–6880.
43. Colucci S, Brunetti G, Oranger A, Mori G, Sardone F, Specchia G *et al.* Myeloma cells suppress osteoblasts through sclerostin secretion. *Blood Cancer J* 2011; **1**: e27.
44. Sottnik JL, Dai J, Zhang H, Campbell B, Keller ET. Tumor-induced pressure in the bone microenvironment causes osteocytes to promote the growth of prostate cancer bone metastases. *Cancer Res* 2015; **75**: 2151–2158.
45. Giuliani N, Ferretti M, Bolzoni M, Storti P, Lazzaretti M, Dalla Palma B *et al.* Increased osteocyte death in multiple myeloma patients: role in myeloma-induced osteoclast formation. *Leukemia* 2012; **26**: 1391–1401.
46. Delgado-Calle J, Bellido T, Roodman GD. Role of osteocytes in multiple myeloma bone disease. *Curr Opin Support Palliat Care* 2014; **8**: 407–413.
47. Bidwell BN, Slaney CY, Withana NP, Forster S, Cao Y, Loi S *et al.* Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. *Nat Med* 2012; **18**: 1224–1231.

48. Noonan K, Marchionni L, Anderson J, Pardoll D, Roodman GD, Borrello I. A novel role of IL-17-producing lymphocytes in mediating lytic bone disease in multiple myeloma. *Blood* 2010; **116**: 3554–3563.
49. Dhodapkar KM, Barbuto S, Matthews P, Kukreja A, Mazumder A, Vesole D *et al.* Dendritic cells mediate the induction of polyfunctional human IL17-producing cells (Th17-1 cells) enriched in the bone marrow of patients with myeloma. *Blood* 2008; **112**: 2878–2885.
50. Zhang Q, Liu S, Ge D, Zhang Q, Xue Y, Xiong Z *et al.* Interleukin-17 promotes formation and growth of prostate adenocarcinoma in mouse models. *Cancer Res* 2012; **72**: 2589–2599.
51. Bian G, Zhao WY. IL-17, an important prognostic factor and potential therapeutic target for breast cancer? *Eur J Immunol* 2014; **44**: 604–605.
52. Taipaleenmaki H, Browne G, Akech J, Zustin J, van Wijnen AJ, Stein JL *et al.* Targeting of Runx2 by miR-135 and miR-203 impairs progression of breast cancer and metastatic bone disease. *Cancer Res* 2015; **75**: 1433–1444.
53. Ell B, Mercatali L, Ibrahim T, Campbell N, Schwarzenbach H, Pantel K *et al.* Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis. *Cancer Cell* 2013; **24**: 542–556.